

RECEIVED
CENTRAL FAX CENTER

T. Irimura et al.
U.S. Serial No. 10/524,970
Page 2 of 13

AUG 09 2007

In the Specification:

Please amend the specification as shown:

Please delete the paragraph on page 5, line 22, to page 6, line 3, and replace it with the following paragraph:

Fig. 2 shows the nucleotide sequence of cDNA (SEQ ID NO: 1) encoding MAH and its predicted amino acid sequence (SEQ ID NO: 2). In the amino acid sequence in Fig.2, the position of loop D is shown by the third underline (lowest underline), and in this loop, particularly 5 amino acids considered to exert a significant influence on binding to sugar chains are enclosed with a square. In the figure, I indicates an Xba I (ctcgag) restriction enzyme site, II indicates a Bgl II (aggatc) restriction enzyme site, and III indicates an Spe I (actagt) restriction enzyme site.

Please delete the paragraph on page 6, lines 7-8, and replace it with the following paragraph:

Fig. 4 is an illustration of varieties of IgA1 sugar chain structures (SEQ ID NO: 34).

Please delete the paragraph on page 6, lines 9-10, and replace it with the following paragraph:

Fig. 5 shows the amino acid sequence of MAH loop C wherein the position of amino acid inserted by modification is indicated (SEQ ID NOS 35-37, respectively in order of appearance).

Please delete the paragraph on page 6, line 11, and replace it with the following paragraph:

Fig. 6 shows an outline of a phage display lectin library (SEQ ID NOS 38-39, respectively in order of appearance).

Please delete the paragraph on page 6, lines 20-23, and replace it with the following paragraph:

Fig. 9 shows amino acid sequences (SEQ ID NOS 38, and 22-31, respectively in order of appearance) of lectins contained in a lectin library used in discrimination of IgA different in sugar chain glycoform and in discrimination of cell subgroup derived from mesenchymal stem cells.

T. Irimura et al.
U.S. Serial No. 10/524,970
Page 3 of 13

Please delete the paragraph on page 7, lines 8-9, and replace it with the following paragraph:
Fig. 15 shows the amino acid sequence of MAII loop D, wherein the position of amino acid inserted by modification is indicated (SEQ ID NO: 40).

Please delete the paragraph on page 21, lines 11-22, and replace it with the following paragraph:
After it was confirmed that MAII lectin was expressed on phage to agglutinate erythrocyte, the sugar chain recognition site of loop C in MAH was modified randomly by AmpliTaq Gold DNA polymerase (manufactured by PE Biosystems) in a Perkin-Elmer 2400 thermal cycler. The primer and reverse primer used therein are shown in Table 1.

Table 1. Primers

Primer (containing an Eco RI site)

5' -

CCGGAATTCGACACTTACNNKNNCATNNKNNKGATNNKNNKGACCCAACTACAG
ACATATC-3' (SEQ ID NO: 32)

Reverse primer (containing a Bam HI site)

5'-CACAAACGAATGGGGATCCAC-3' (SEQ ID NO: 19)

Please delete the paragraph on page 23, lines 15-23, and replace it with the following paragraph:

Each modified MAH cDNA-pComb3 was subjected to PCR with a sense primer N-Flag-XhoI (5'- CCAGGTGAAACTGCTCGAGTCAGATG-3', SEQ ID NO: 20) and an antisense primer N-Flag-BglII (5'- TCCACCGCCAGATCTCTATGCAGTGTAAACG-3', SEQ ID NO: 33). The resulting PCR product was recovered with a PCR Purification Kit (manufactured by QIAGEN) and treated with restriction enzymes Xho I And Bgl II. The product thus treated was ligated to XhoI/BglII-digested pFlag-ATS (manufactured by Sigma). Each plasmid thus obtained was incorporated into E. coli JM109.

Please delete the paragraph on page 35, lines 6-15, and replace it with the following paragraph:

T. Irimura et al.
U.S. Serial No. 10/524,970
Page 4 of 13

Table 5. Preparation of the vector

Composition of the PCR reaction solution (template (pFLAG-ATS), 1 µl; primers (pFLAG-Spc I-sense 100 ng/µl and pFLAG-Spc I-anti 100 ng/µl), each 1.25 µl; 10 x PCR buffer, 5 µl; dNTP, 1 µl; Milli Q, 40.5 µl; and psu turbo, 1 µl)

PCR reaction conditions (95°C for 30 sec and 12 cycles [95°C for 30 sec, 55°C for 1 min and 68°C for 10 min])

Primer sequences

pFLAG-Spc I-sense: 5' - ccgggtaccgcactagtagatagatgagctc (**SEQ ID NO: 3**)

FLAG-Spc I-anti: 5' - gagctcatctatctactatgtcgaggtaaccgg (**SEQ ID NO: 4**)

Please delete the paragraph on page 35, line 25, to page 36, line 11, and replace it with the following paragraph:

Table 6. Transformation with the vector

Composition of the PCR reaction solution (template 1.5 µl; primers (N-Flag-Xhol, 100ng/µl and MAH-SpcI-anti, 100 ng/µl, each 0.5 µl; dNTP, 4 µl; 10 x PCR buffer, 5 µl; Taq Gold, 1 µl; and Milli Q 38.5 µl)

The PCR reaction conditions were general (96°C for 5 min, 30 cycles [96°C for 1 min, 55°C for 1 min, 72°C for 2 min] and 72°C for 5 min)

Primer sequences

pFLAG-XhoI: 5' - ccaggcgaaactgctcgagtcaaglg (**SEQ ID NO: 5**)

MAH-Spc I anti: 5' - tgggcaactagttgcagtgtacgtgcg (**SEQ ID NO: 6**)

Primer sequences used in sequencing

N-26: 5' - catataacggtctggcaaatttc (**SEQ ID NO: 7**)

Loop D-Seq: 5' - gttaatagcatcttagttaccc (**SEQ ID NO: 8**)

T. Irimura et al.
U.S. Serial No. 10/524,970
Page 5 of 13

Please delete the paragraph on page 36, line 21, to page 37, line 27, and replace it with the following paragraph:

A multicloning site Bgl II site could be converted into Sph I site. There was no mutation other than the target region. Theoretically predicted 120 modified MAHs could be isolated and identified.

Table 7. Primers used in random modification

N-Flag-Xhol: 5'-ccaggtgaaactgcgtcgaggtagatg (**SEQ ID NO: 5**)

LLD3: (**SEQ ID NO: 9**)

5'-ctacaagatctaacatcgltgggtttcaactgcnnnnltaggagcacccgtggcagcaga

LLD4: (**SEQ ID NO: 10**)

5'-ctacaagatctaacatcggtgggtttcaacigcillmnnaaggagcacccgtggcagcaga

LLD5: (**SEQ ID NO: 11**)

5'-ctacaagatctaacatcggtgggtttcaactgcgtttaggmnnnacccgtggcagcaga

LLD6: (**SEQ ID NO: 12**)

5'-ctacaagatctaacatcggtgggtttcaacigcgtttaggagcmnnnacccgtggcagcaga

Individually designed primers

MAH loop D-1Phe: (**SEQ ID NO: 13**)

5'-ctacaagatctaacatcggtgggtttcaaaaaactgcgtttaggagcacccgtggcagcaga

MAH loop D-2Asp: (**SEQ ID NO: 14**)

5'-ctacaagatctaacatcggtgggtttcaacatcgctttaggagcacccgtggcagcaga

MAH loop D-3Cys: (**SEQ ID NO: 15**)

5'-ctacaagatctaacatcggtgggtttcaacigcacatitaggagcacccgtggcagcaga

MAH loop D-4Asp: (**SEQ ID NO: 16**)

5'-ctacaagatctaacatcggtgggtttcaactgcacatitaggagcacccgtggcagcaga

MAH loop D-6Phe: (**SEQ ID NO: 17**)

5'-ctacaagatctaacatcggtgggtttcaactgcgtttaggagcaaaacccgtggcagcaga